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THE DEVELOPMENT OF GREGARINES AND THEIR RELATION TO THE HOST TISSUES. (III) IN *GREGARINA RIGIDA* (HALL) ELLIS

MINNIE WATSON KAMM

This paper is the third of a series in which it is desired to show the effect of gregarines upon the host cells which they parasitize. In the two former papers the species considered were intracellular parasites, the young stages of which live within the intestinal cells and absorb their entire nourishment therefrom; they are consequently disastrous in their effect upon these cells. In *Cephaloidophora delphinia* (Watson) Kamm (1918) the minute trophozoite soon outgrows the parasitized cell and absorbs the walls of this and the adjoining cells, and finally comes to occupy considerable space within the epithelium, being highly deleterious in its action upon the host tissue. A moderate infection with this parasite therefore causes a serious gregarinosis in the host. It is questionable whether the host, the large white sand-flea *Talorchestia longicornis* (Say), is able to regenerate new tissue to replace the lost.

The first parasite studied (Kamm, 1917), *Stenophora lactaria* Watson, injures its host the milliped *Callipus lactarius* (Say), in much the same manner but to a less serious degree.

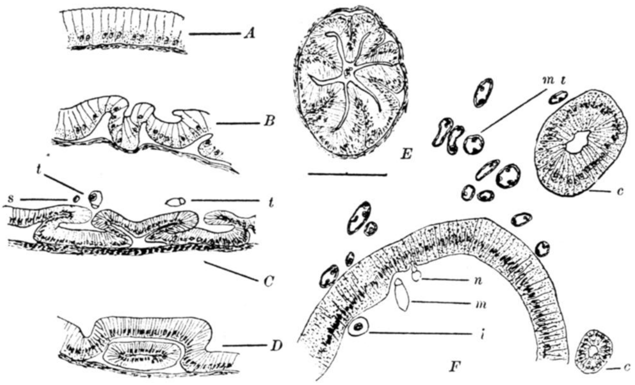
The present species, *Gregarina rigida* (Hall) Ellis, parasitizes species of the Acridiidae, the present specimens being taken from *Melanoplus differentialis* (Uhler). It is a particularly good species for sectioning because the percentage of infection runs high and the number of parasites per host is large.

The entire alimentary tract (Fig. 1) was sectioned in order to determine the histology of the cells in the various regions and the degree of parasitism involved, this degree being dependent upon the character of the cells. The walls of the crop are chitinous and armed with teeth and are therefore not adapted to parasitism. The thin-walled, highly vascular mid-intestine is, on the contrary, well adapted to the existence of gregarines; I have found as many as fifty-four parasites in one cross-section made through the anterior portion of this region.

The six pyloric ceca originate near the anterior end of the mid-intestine (Text figs. A-F) and pass both forward and back, the backwardly-directed portions being only about one-third as long and much more slender than those directed anteriorly. They are admirably adapted to the early requirements of the parasite. The tiny sporozoites find lodging here without danger of currents of food or strong muscular contractions tearing them loose from their hold on the cells. Some unilocular stages are to be encountered in the mid-intestine walls but by far the larger number is situated here. Consequently the larger epimerited trophozoites and smaller sporonts are abundant here also.

The Malpighian tubules which open into the alimentary tract at the junction of mid-intestine and intestine proper are not parasitized with gregarines.

The anterior end of the intestine proper is similar histologically to the mid-intestine and contains some gregarines but the rectum, which is chitinous-walled, is free.



Text Figure A-F.—*A, B, C,* and *D* represent the origin of the ceca at the junction of crop and mid-intestine. *A,* cell wall near posterior end of crop. *B,* convolutions in wall. *C,* double folds in wall, cells small, several gregarines near folds, *t* small sporonts, *s* sporozoite, moving freely from *c* ceca to intestine. *D,* cecum closed off from intestinal epithelium but still within longitudinal muscular layer. It soon penetrates this wall and lies freely in the coelom. *E,* cross-section through pyloric orifice of host between crop and mid-intestine. *F,* cross section through anterior end of mid-intestine and through the small backwardly-directed ceca (*c*) and the anterior end of the Malpighian tubules (*mt*); one trophozoite (*n*) is attached to the cell-wall, one small sporont (*m*) is cut longitudinally and a larger one (*i*) crosswise.

All drawings were made at same magnification, the line (under *E*) representing 200μ.

Sections were cut thin (5μ) so as to include portions of the same young parasite in several successive sections. Stains used included Delafield's and iron hematoxylin countered with orange G; best results were obtained with the early stages by using the latter but for the larger parasites the former was the more satisfactory. In order to determine the effect of the parasite upon cells, both stains were useful, the iron stain for the chromatin and Delafield's for cytoplasmic modifications if any.

Free sporozoites (Fig. 2) were found in large numbers in both mid-intestine and ceca. Much to my surprise they are spherical or sub-spherical in shape, measuring 14 to 20μ in diameter, the nucleus being proportionately large and filled with scattered granules of chromatin.

Upon coming in contact with the epithelium, there is pushed out from the sporozoite a small conical protuberance (Fig. 3) at first devoid of endoplasm. This papilla elongates into a slender neck, often as long as the sporozoite itself, and forces itself through the intima into the wall of the intestine. This spherical sporozoite and its ameboid character have not to my knowledge been heretofore reported for gregarines but from many hundreds of observations with high power, I am convinced that these phenomena exist. The protuberance has been seen in its incipiency through the gradually growing stage when the sporozoite lies adjacent to the wall and to the penetration of the long, now pyriform, trophozoite which becomes firmly attached.

In none of the observations made was the cell-wall punctured, the sporozoite pushing up between two cells and splitting them apart instead. This is obviously the easier process for the apex is unarmed and yet it affords a holdfast, which is all the young parasite desires. The point often enters the cell area at considerable slant and the parasite is unable to recover its normal perpendicular position, possessing a crooked epimerite during the rest of its trophozoitic life.

Sporozoites are generally found in the recesses between folds of the ceca where they collect in groups and are fully protected. They also sink into recesses which appear to have been made by parasites which have left their holdfasts and become free-living sporonts. The more exposed regions are more infrequently parasitized because the sporozoites which have endeavored to attach themselves here have been swept away.

Very many faintly-staining bodies the size and shape of sporozoites are often found massed along the periphery of the lumen, their nuclei generally disintegrated and their outline often indefinite. I venture the suggestion that these are dead or infertile sporozoites; and that sporozoites must be eaten by the proper host within a certain time, probably a few weeks after extrusion from the cyst, in order to remain fertile. The similarity in size and shape preclude the possibility of their being sporozoites of other gregarines not infective in this particular host.

When the process of the sporozoite is first thrust into the cell-area it is ectoplasmic but it soon fills with endoplasm and swells into a knob-like holdfast not easily dislodged (Fig. 5). A septum begins to develop although not at first visible but evident from the differential staining. The trophozoite has now fully established itself and is growing rapidly. It has crowded several cells aside at their apices, not only the epimerite but a goodly portion of the protomerite being pushed up into this cell-area.

An instance in which the entire trophozoite is pushed up into the cell area is described by Léger and Duboscq (1899) for *Gregarina davini* Léger and Duboscq. The parasite has located in a recess between two lobes, its large dilated epimerite occupying the regenerative space at the base of the epithelium, which contains many nuclei but no cell walls. It transforms this space into "un kyste epithélial." The nuclei are massed near the epimerite but are not in the least affected by its presence.

The septum is now established and the staining in the two parts that are characteristic of the sporont; the nucleus is very large and contains many karyosomes which later fuse into one; and the epimerite now contains a small amount of endoplasm only near its base.

If the epithelium were being affected by the parasite this would now be evident. The cells are undoubtedly crowded and their nuclei often pushed out of place and shape; but upon careful observations with both stains I can detect no difference in character between the normal and crowded cytoplasm or between the normal and distorted nuclei. There is no hypertrophy and I do not think the crowding could be called atrophy for the cells return almost to their normal shape. The larger the parasite becomes the less its presence disturbs the cells, for the body retreats into the lumen except for the small epimerite (Figs. 6, 7). I think the cell always retains a trace of the indentation made by the epimerite and that this depression is taken advantage of by the young sporozoites as a new holdfast.

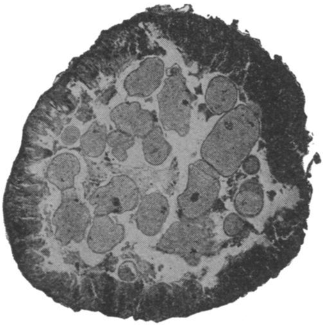
The epimerite is gradually constricted as the parasite grows, until it becomes a perfect sphere, completely pinched off, leaving a small indentation but no open wound in the apex of the protomerite (Fig. 9) which soon smooths out. Vigorous muscular contractions of the intestine or the current of food carries the sporont away and leaves the epimerite in the cells (Fig. 10), where it soon atrophies and disappears.

If its movements are too strenuous or outside mechanical means interfere, the parasite may lose its epimerite prematurely (Fig. 11). I doubt if it is able to recover the loss; it is probable that a thin stream of protoplasm continues to flow from the apex until the protomerite collapses and the animal dies. The parasite is probably unable to regenerate a new epimerite and a new fully developed organ would be unable to penetrate the epithelial cells.

Large free sporonts in the ceca are the exception rather than the rule. A maximum length of 90μ was observed here, the animals soon moving out into the intestine, where more food is to be found. This migration is not due to any new chemical affinity which the parasite acquires, for the weak acid secreted by the gastric ceca passes directly into the mid-intestine, where the parasites continue to be bathed in it; the migration is unexplainable. The fact that the ceca become too small is not adequate explanation.

In the intestine the sporonts are tightly compressed between food-masses the greater part of the time, since the host eats frequently, and they are often flattened so that in cross-section one dimension is three times the other. The parasites just as readily adhere to food masses as to the intestinal walls, which means that they are frequently carried to the exterior in food-pellets.

Two sporonts in association preparatory to the formation of a cyst are shown in the microphotograph (Text fig. G).



Text Figure G.—Microphotograph of section through mid-intestine showing lumen almost completely filled with parasites cut at various angles, the short wavy outlines denoting movements when fixed. Four associations of adult sporonts are shown.

In comparison with the number of parasites encountered, the number of cysts is very small, so the gregarines which are able to complete their life-history are comparatively few in number.

One more paper will be presented in this series, on another epimerited species of a genus not closely related to the genus *Gregarina* and a bibliography will be given covering work on Effect on Host Tissues.

SUMMARY

1. Ten successive stages in the life-history of *Gregarina rigida* (Hall) Ellis are shown from the spherical sporozoite free in the lumen of the intestinal tract to the associative sporonts.

2. This species possesses an epimerite which develops in the sporozoite as an ameboid papilla, becoming a long slender neck, which is thrust through the intima between two epithelial cells rather than into one, where it obtains a holdfast and develops at the cell-apex a rounded knob for an epimerite.

3. The cells parasitized are affected only mechanically, being pushed aside during the parasite occupancy; no chemical effect upon the cell is noted at any stage of occupancy and the cell is apparently uninjured.

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Kamm, Minnie Watson. 1917.—The Development of Gregarines and Their Relation to the Host Tissues: (I) in *Stenophora lactaria Watson.* Jour. Parasit., 3:124-30.

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Explanation of Plates

All drawings except Fig. 1 were made with camera lucida from sections cut at 5μ. The line in drawings 2, 3, 4, and 5 represents 10μ, in the other drawings 100μ.

Explanation of Plate IV

Fig. 1. Alimentary tract of *Melanoplus differentialis* (Uhler), after Folsom.

p pharynx
o esophagus
gl salivary glands
a crop
g gastric ceca
m stomach or mid-intestine
mt Malpighan tubules
i intestine
j colon
r rectum

Fig. 2.—Two sporozoites free in cecum.

Fig. 3.—Sporozoite with apical protuberance developed ready for penetration into cell area. See also Fig. 13.

Fig. 4.—Portion of cecum with three sporozoites in the process of entering the cellular area, two sharply pointed and one with blunter apex. One sporozoite with elongated protuberance lies near the epithelium. See also Figs. 12 and 13.

Fig. 5.—Slightly knobbed epimerite in young trophozoite in which a difference in staining reaction is discernible between protomorite and deutomerite.

Fig. 6.—A large trophozoite crowded between two lobes of the cecum.

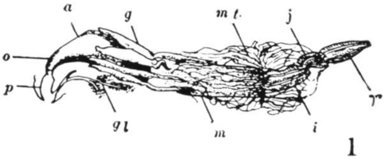
Fig. 7.—A large trophozoite in epithelium with its epimerite somewhat eccentric.

Fig. 8.—Trophozoite with epimerite and septum developed. The epimerite is held in place by pressure from the outside rather than by a specialized holdfast.

Fig. 9.—Sporont with indentation at apex where epimerite has recently become detached.

Fig. 10.—Sporont which has just become constricted from its epimerite.

Fig. 11.—Trophozoite which has become prematurely severed from its epimerite.



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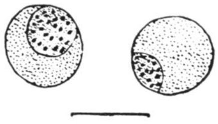

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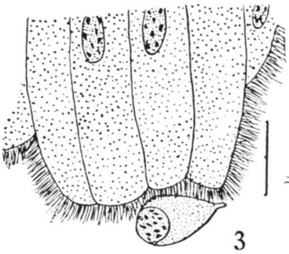

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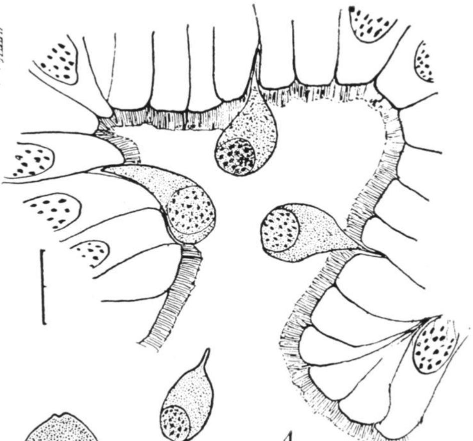

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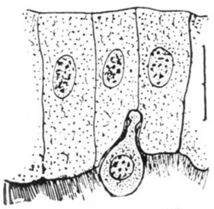

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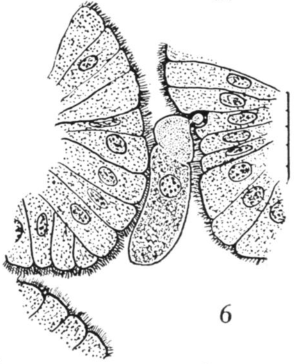

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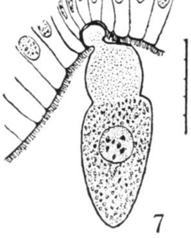

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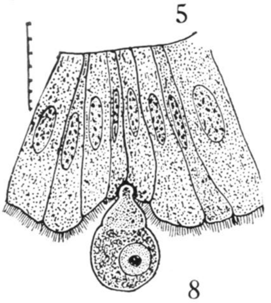

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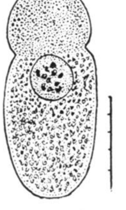

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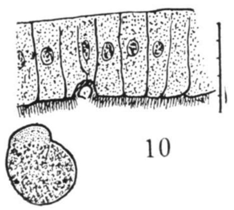

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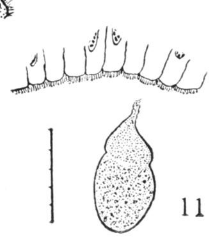

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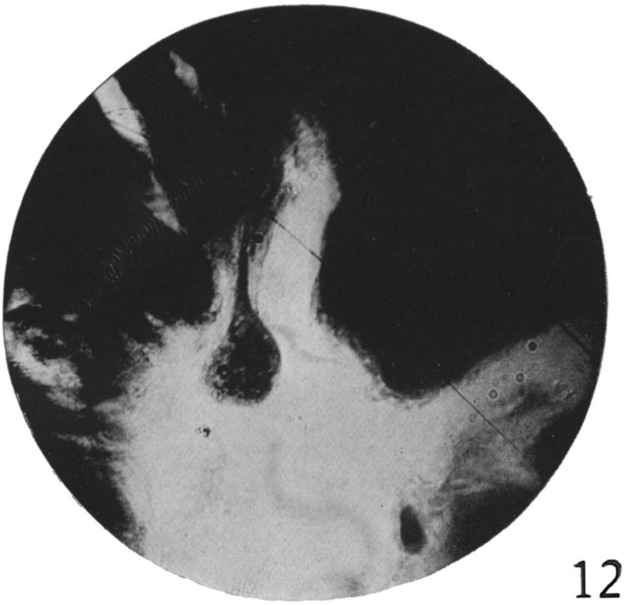
PLATE IV

EXPLANATION OF PLATE V

Fig. 12.—Microphotograph (× about 1200) showing pyriform sporozoite about to penetrate into area between cells. Note large nucleus upon face of cell.

Fig. 13.—Microphotograph (× about 1200) showing three sporozoites, one at right, with protuberance half developed, lying near epithelium; other two with apical ends at lower focus than body of cell but showing the rotund character of the sporozoite at its distal portion, and the large nucleus which contains many distributed karysosomes.



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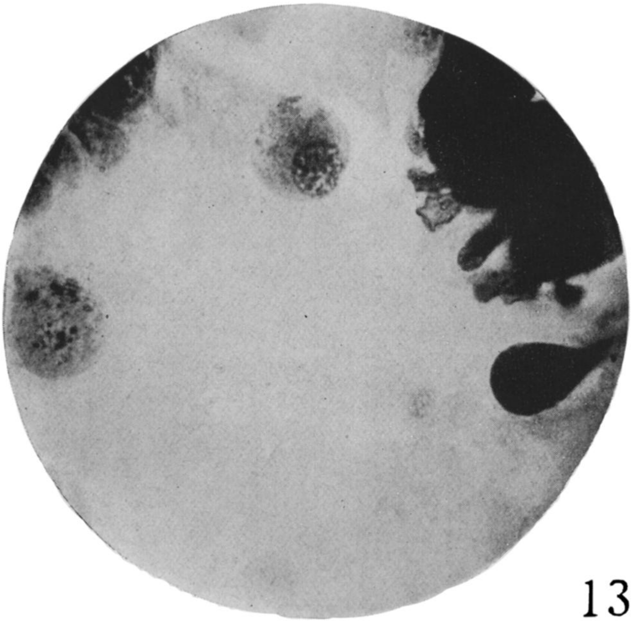

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PLATE V